Engineering Microfabricated Pillars to Create a Physiologically Relevant Model of 3D Cancer Culture

Vivek Somasundaram[1], Davi da Silva[1] Nicole Y. Morgan[1], Tom Pohida[2], , Marcial A Garmendia-Cedillos[2], Joanna Poprawski[3],Michael M. Gottestman[3]

[1]National Institute of Biomedical Imaging and Bioengineering, [2]Center for Information Technology, 3National Cancer Institute , National Institutes of Health

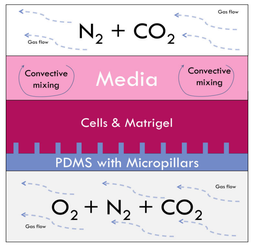
Introduction

Methods to culture cancer cells to closely model physiological conditions are essential to test the efficacy of treatments and to learn more about how cancer behaves in vivo. Animal studies are expensive, and often possess many unknown variables, affecting its ability for translation to clinic. In current 3D spheroid culture, spheroid diameters are limited to about 400-600 µm before central hypoxia occurs. Central hypoxia alters cell physiology and makes it difficult to model conditions in vivo. This project investigates the effects of a bioreactor system that utilizes PDMS micro pillars for oxygen delivery. Oxygen transmission was achieved by a gradient created by adding oxygenated gas below the pillars and maintaining anoxic conditions above the pillars, creating a desirable model for vascular tumors.

Objectives

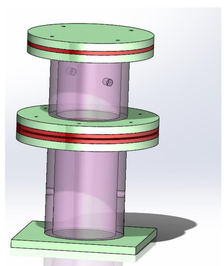
- To improve the current design of the micro fabricated membrane molds to increase sub well thickness precision
- To develop quality control tests that assure the membranes undergo cell experiments successfully
- To block oxygen transmission through the base of the pillars
- Expand the single chamber design to a six well plate design

Bioreactor Schematic



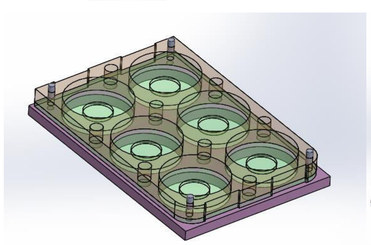
The image on the left is a schematic of the bioreactor during a cell experiment. An oxygen gradient is created that promotes oxygen flow from the bottom of the chamber through the PDMS membrane and micropillars and cells and media. This set up was used to culture cells in the gradient experimental group.

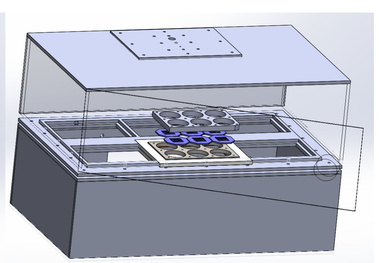
Bioreactor Designs



The earlier single chamber design(left) was first used for cell experiments.

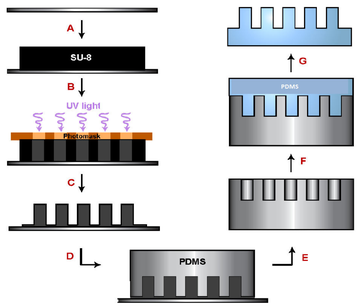
This design was eventually expanded to a six well plate design(bottom, left) , that was placed in a gas chamber(bottom, right) in order to create the necessary oxygen gradient.



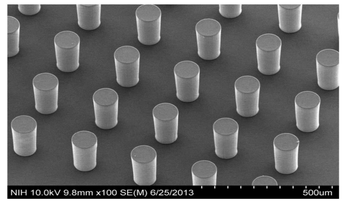


Pillar Microfabrication

The pillars for oxygen delivery were fabricated using photolithography and then PDMS to create a negative impression of the pillars. First, a light sensitive polymer(photoresist SU-8) was placed on a silicon wafer and then subjected to a pattern of light determined by the photomask**(A-B)**. Unexposed polymer was then dissolved away and PDMS was poured, cured, and unmolded to create a negative impression of the pillars**(C-E)**. Finally PDMS is poured onto this mold to create the final micropillars**(F-G, SEM below, right)** The pillar dimensions*(below)* were used to mimic capillary dimensions *in vivo (below)*.

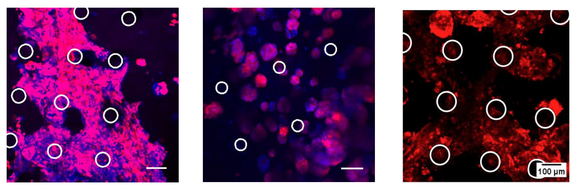


Pillar Dimensions	
Diameter	~100 µm
Spacing (edge-to-edge)	~200 µm
Height	~250 µm



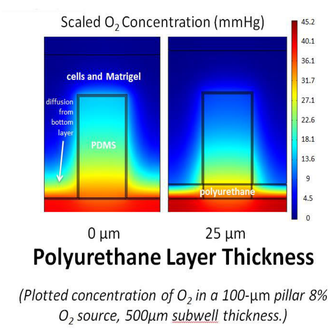
Results: Cell Morphology

3% O_2 gradient (old system) | 3% O_2, no gradient (old system) | 3% O_2 gradient (new system)



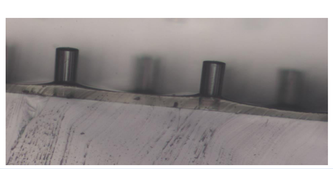
OVCAR8-dsRed2 cells with Hoechst(left, center) and without(right) with both the old and new bioreactor designs with the silicon hydrogel pillars after 7 days of culture. Distinct differences in morphology are seen between both the gradient groups and the groups without a gradient. The pillars are outlined in white and the scale bar present represents 100 microns on the images taken as maximum intensity projections on a confocal z stack.

Results: Oxygen Block



(Plotted concentration of O_2 in a 100-µm pillar 8% O_2 source, 500µm subwell thickness.)

As seen in the graph, bare PDMS allows significantly more diffusion than when a 25 micron thick later of polyurethane deposited. Blocking oxygen ensures further replication of physiological conditions.



Results: Subwell Thickness Precision

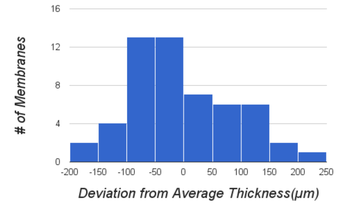
The previous mold design for the membranes used in the bioreactor had a subwell thickness range of about 100µm from the desired 500µm thickness. To mediate this, a new mold design that involved plasma bonding the plugs to a glass substrate was utilized instead of gluing the microfabricated plugs to the substrate. The result was more precise subwell thicknesses within a membrane*(right)*, despite using plugs that varied the same in thickness.

Furthermore a COMSOL simulation was run to model how the PDMS thickness below the pillars impacts oxygen consumption. Using a Michaelis-Menten model of cell oxygen consumption:

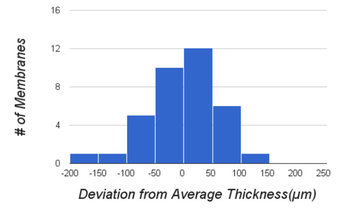
$$\left(R = R_{\max}\frac{c_{O_2}}{c_{O_2}+K_{MM}}\right)$$

the oxygen concentration was modeled with an 8% oxygen gradient(shown right). This graph enabled calculations to estimate that the variability of oxygen diffusing through the membrane has ultimately been reduced from 22% to approximately 12%, as illustrated by the narrower dark blue band.

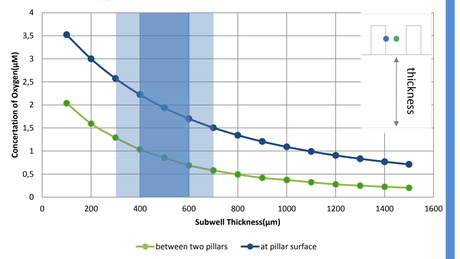
Old Membrane Mold



New Membrane Mold



Oxygen Concentration vs Subwell Thickness



Conclusions & Future Work

Conclusions

- PDMS micropillars effectively mimic oxygen transmission to cells in vivo
- A new mold design increases subwell thickness precision and consistency of oxygen transmission within a membrane

Future work

- Perform more cell experiments to test efficacy of drugs on cancer spheroids grown in the bioreactor
- Refine a method to block oxygen transmission around the base of the pillars
- Test to determine optimal matrigel thickness for cancer cell culture in bioreactor
- Extend bioreactor experiments to other cell types

Acknowledgements

This research was funded by the Intramural Research Programs at the National Institute of Biomedical Imaging and Bioengineering, as well as the Biomedical Engineering Summer Internship Program

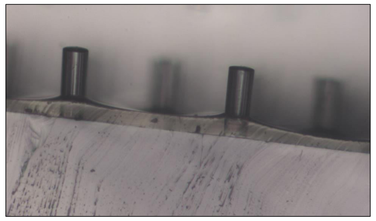
References

1. Jaeger AA, Das CK, Morgan NY, et al. Microfabricated polymeric vessel mimetics for 3-D cancer cell culture. *Biomaterials*. 2013;34(33):8301-8313. doi:10.1016/j.biomaterials.2013.07.013.

Bioreactor Quality Control

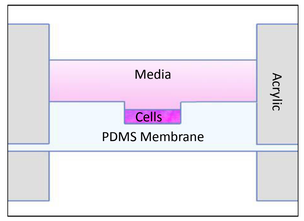
Leakage Testing

Media leakage would compromise a seven day experiment, necessitating the addition of a leakage test that each membrane must pass. In addition to testing the quality and integrity of the membrane, this test also evaluates the quality of the acrylic sandwiches the membranes are place in**(right).** Ethanol was used to test the membranes as it wets the hydrophobic surface of the silicon hydrogel better than water does, enabling more stringent quality control standards.

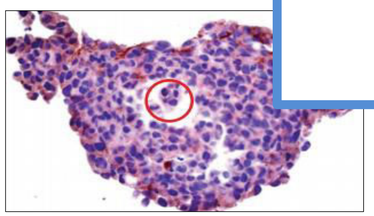


Oxygen Blocking

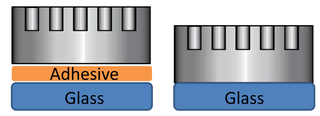
As it is desirable to block oxygen transmission through the area around the base of the pillars to more accurately model physiological conditions. To pursue this, a deposition method of adding a polyurethane mixture in chloroform/DMF solvent. This mixture was then vacuum baked to evaporate the solvent and even spreading of the polymer**(left).**



Subwell Thickness Measurements		
	Averages	Oxygen variation at Pillars
Old Design	668±99 µm	22%
New Design	551±62 µm	12%



seen in the image, cells become hypoxic at approximately 100 microns from the pillar(outlined in red), replicating conditions in vivo.



Bioreactor Chamber Set-up

Cross section of bioreactor sandwich